

Role of Endomyocardial Biopsy in Rejection Surveillance After Heart Transplantation in Neonates and Children

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Objectives. The aim of this study was to retrospectively evaluate the sensitivity of noninvasive surveillance (physical examination, echocardiography) of rejection in accurately predicting histologically documented rejection episodes. Additionally, the usefulness of routine scheduled biopsy and its safety in pediatric patients was explored.

Background. Endomyocardial biopsy has been utilized as the standard for rejection surveillance after heart transplantation in adults, but its role in documenting clinically suspected rejection and in routine surveillance of pediatric patients has not been agreed upon.

Methods. Heart transplantation was performed in 14 neonates and 21 children. The immunosuppressive regimen consisted of cyclosporine, azathioprine and prednisone. All patients underwent routine noninvasive rejection surveillance that included clinical examination and echocardiography. In the neonates, biopsy was performed quarterly beginning 6 months after transplantation, after cessation of prednisone therapy. In the children, biopsy was performed 15 times in the 1st year. A minimum of five

biopsy samples were interpreted using the Working Formulation for Heart Transplant Rejection.

Results. In the neonates, 37 biopsies were performed. Evidence of rejection was present in only three biopsy samples obtained during eight episodes (38%) of clinically suspected rejection. In 29 biopsies performed when rejection was not clinically suspected, each biopsy was free of cellular infiltrate. In the children, 291 biopsies were performed. Evidence of rejection was present in only seven biopsies (41%) from 17 episodes of clinically suspected rejection. Cellular rejection was discovered during routine rejection surveillance biopsies in asymptomatic patients in 23 (8.4%) of 274 biopsies.

Conclusions. In neonates with clinically suspected rejection, endomyocardial biopsy identified which patients did not require rejection therapy. Endomyocardial biopsy surveillance did not detect any unsuspected cases of rejection. In children, noninvasive rejection surveillance was less reliable even in asymptomatic patients, suggesting that periodic endomyocardial biopsy should be utilized.

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Despite improved immunosuppression, the early and accurate diagnosis of acute rejection after heart transplantation remains a challenge (1-7). Previous reports (8-12) demonstrating the utility of endomyocardial biopsy for reliably detecting cellular rejection have established the biopsy as the standard for rejection surveillance after heart transplantation in adults. This experience, dating back to 1972, comes from the Stanford University Heart Transplant Program, which began invasive rejection surveillance using the bioprobe designed by Caves et al. (13), with interpretation of tissue by Billingham (8). Endomyocardial biopsy has not been widely utilized in infants and young children for many

reasons including the proposed need for general anesthesia, risk of ventricular perforation and thrombosis of access vessels and poor myocardial sampling yield (14,15). In addition, some investigators (14) believe that rejection can be detected noninvasively without need for biopsy sampling.

The objectives of this study were 1) to retrospectively evaluate the sensitivity of noninvasive rejection surveillance (physical examination and echocardiography) in accurately predicting histologically documented rejection episodes; 2) to evaluate the usefulness of routine scheduled biopsies; and 3) to evaluate the safety of endomyocardial biopsy in pediatric patients.

Methods

Patients. Between May 1988 and November 1991 we performed orthotopic heart transplantation in 35 patients. Group I consisted of 14 neonates; the diagnosis was hypoplastic left heart syndrome in 13 and aortic stenosis with severe endocardial fibroelastosis in 1. The mean age at transplantation was 19 days (range 2 to 49). Group II

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consisted of 21 children; the diagnosis was cardiomyopathy in 16, postoperative congenital heart disease with refractory ventricular failure in 4 and chronic rejection requiring a second heart transplantation in 1. The mean age at transplantation was 7 years (range 1 to 17).

Immunosuppressive regimen. The posttransplantation immunosuppressive regimen was based on triple therapy with cyclosporine, azathioprine, and prednisone. Cyclosporine was administered orally two or three times a day to maintain trough whole blood concentration measured by high pressure liquid chromatography >150 ng/ml in the neonatal patients and ≥ 200 ng/ml in children. Azathioprine treatment was started at 2 mg/kg body weight per day and titrated to a white blood cell count of $5,000/\text{mm}^3$. Prednisone dosage was begun at 1 mg/kg per day for the 1st 2 weeks and was gradually decreased to 0.2 mg/kg per day by 8 weeks after transplantation. In Group I, efforts were made to discontinue prednisone at 6 months as guided by endomyocardial biopsy findings.

Acute cellular rejection (grade ≥ 2 International Society of Heart Transplantation) (16) was treated with 10 mg/kg intravenous methylprednisolone daily for 3 days. OKT3 (Ortho) was administered at 2.5 to 10 mg/dose for 7 to 14 days for the management of rejection refractory to steroid pulse therapy or associated with ventricular dysfunction.

Rejection surveillance. *Noninvasive rejection surveillance.* Clinical assessment included nursing and parental accounts of heart rate and of changes in the patient's activity or appetite, or both. Reduced activity or appetite prompted further investigation. Atrial or ventricular ectopic activity or tachycardia at rest, defined as an increase in heart rate of 15 to 20 beats/min above the normal baseline rate at rest, was considered suspicious for rejection and an echocardiogram was performed.

Routine echocardiographic studies in all patients were performed twice weekly in the 1st month, weekly in the 2nd month and monthly thereafter through the end of the 1st year after transplantation. An echocardiogram was also employed whenever rejection was clinically suspected. Echocardiography measured left ventricular dimensions, posterior wall thickness and shortening fraction. Acute left ventricular end-diastolic dimension increases of 20%, left ventricular posterior wall increases of 20% and left ventricular shortening fraction decreases of 20% were deemed significant. The presence of a pericardial effusion or new valvular regurgitation provided supportive data for rejection.

Invasive rejection surveillance. In Group I routine quarterly biopsies were performed beginning 6 months after transplantation and continuing until 18 months after transplantation, and then with each annual comprehensive catheterization. During the 1st 6 months after transplantation, biopsy also was performed when clinically suspected rejection episodes did not resolve after one course of antirejection therapy. In Group II, routine biopsies were performed weekly for the 1st month, biweekly in the 2nd and 3rd months and monthly until the 9th month. Biopsies were

performed quarterly thereafter until 18 months after transplantation and then with each comprehensive annual catheterization. Biopsy was also performed if rejection was clinically suspected. If any biopsy sample demonstrated rejection, serial biopsy samples were evaluated until the episode clearly resolved.

Biopsy technique. All biopsies were performed on an outpatient basis after hospital discharge after transplantation. In patients <5 years of age, light sedation was established with short-acting intravenous fentanyl/droperidol or midazolam, and local anesthesia was provided with lidocaine. In patients >5 years of age, no sedatives were administered, but adequate local anesthesia was obtained with lidocaine. All patients received heparin (50 U/kg).

After venous access and measurement of right heart pressures and cardiac index, a 6F Mullins transeptal sheath (USCI) was advanced over a balloon wedge catheter to the right ventricular apex and the pressure transduced through a side-arm sheath. A 5.6F biptome (Mansfield Byceps) with 1.8-mm jaws was utilized to obtain five to eight endomyocardial biopsy samples from different sites along the right ventricular septum and apex.

Safety evaluation. An echocardiogram was performed after the biopsy to exclude hemopericardium, and fluoroscopy of the chest was done to rule out pneumothorax after a jugular approach. Patients were then sent to the outpatient day hospital for 6 h of observation and discharged if the biopsy sample showed no evidence of rejection.

Evaluation of biopsy samples. The tissue analysis of the five to eight endomyocardial biopsy samples consisted of a 4-h short cycle processing technique. Hematoxylin/phloxine/saffarin stain for light microscopy was employed because of its usefulness in identifying old biopsy sites and myocyte damage associated with rejection. Eight histologic sections were carefully reviewed and graded according to the Working Formulation for Heart Transplant Rejection (16).

Statistical methods. To compare clinical surveillance with biopsy, clinically suspected rejection episodes (as defined earlier) were evaluated by biopsy. For these comparisons, the biopsy results were defined as the reference standard and as the unit of analysis (8,11,16). A proportion estimate was computed for the proportion of clinically suspected episodes confirmed by biopsy. To evaluate the utility of periodic surveillance by biopsy, both the proportion of rejection episodes detected in this manner and the proportion of times that periodic surveillance revealed evidence of rejection were computed. Ninety-five percent confidence intervals for each proportion estimate were computed, using the methods of Clopper and Pearson (17). Similar one-sided intervals were computed for safety of biopsy.

Results

Transplantation outcome. In Group I, 9 of 14 neonates had survived at a mean follow-up time of 25 months (range 9 to 36). There were five deaths: three from infection and two

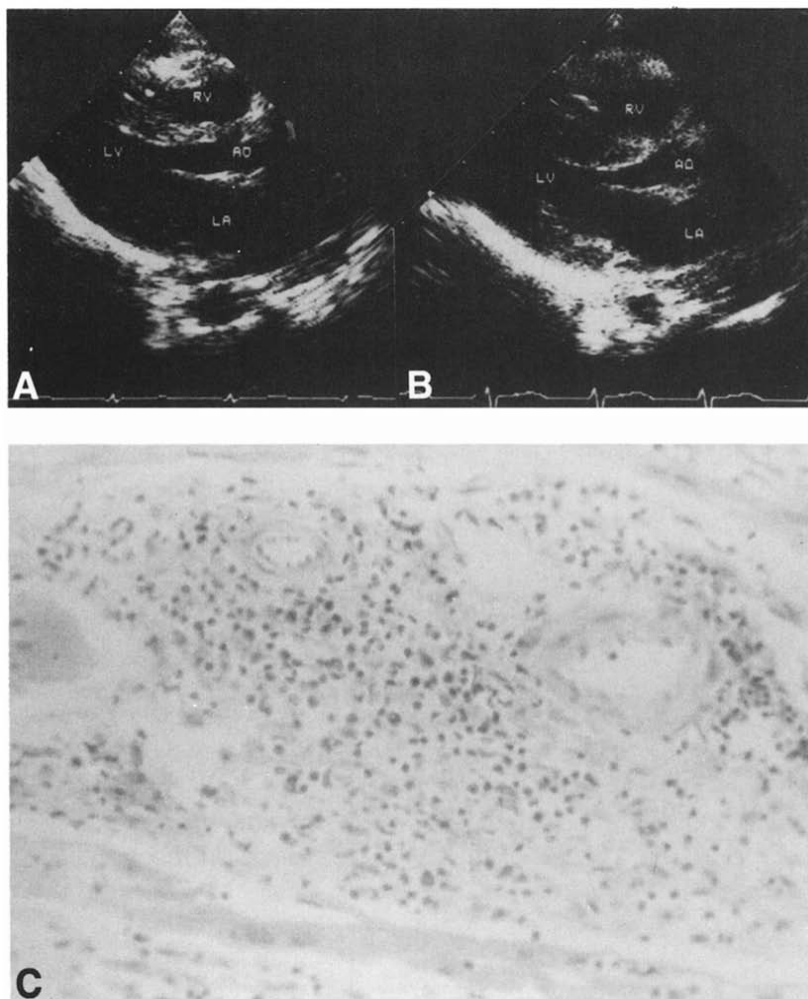


Figure 1. A and B, Two-dimensional parasternal long-axis echocardiograms from a neonate demonstrating (A) normal left ventricular posterior wall thickness (4 mm) and cavity dimensions 2 weeks after heart transplantation and (B) at 5 weeks after transplantation demonstrating an acute increase in left ventricular posterior wall thickness, measuring 8 mm in diameter. C, Same patient. Autopsy specimen after sudden death demonstrating dense lymphocytic infiltrate with severe myocyte necrosis. AO = aorta; LA = left atrium; LV = left ventricle; RV = right ventricle.

sudden deaths from acute cellular rejection at 35 days and 375 days, respectively, after transplantation. (The latter death occurred before initiation of the protocol.)

In Group II, 16 of 21 children had survived at a mean follow-up time of 21.5 months (range 1 to 42). There were five deaths: two from donor graft failure, one from acute cellular rejection 18 days after repeat transplantation (after 2 negative biopsies), one from posttransplantation lymphoproliferative disorder and one from sepsis.

Rejection surveillance. *Group I.* Twelve episodes of rejection occurring in eight neonatal transplant recipients were clinically suspected and treated in the 1st 6 months after transplantation before invasive surveillance began. Clinical and echocardiographic features of the suspected rejection episodes included irritability and rest tachycardia in five episodes, atrial ectopic activity in three episodes, an increase in left ventricular posterior and septal wall thickness in three episodes (Fig. 1) and ventricular dysfunction with poor cardiac output in one episode.

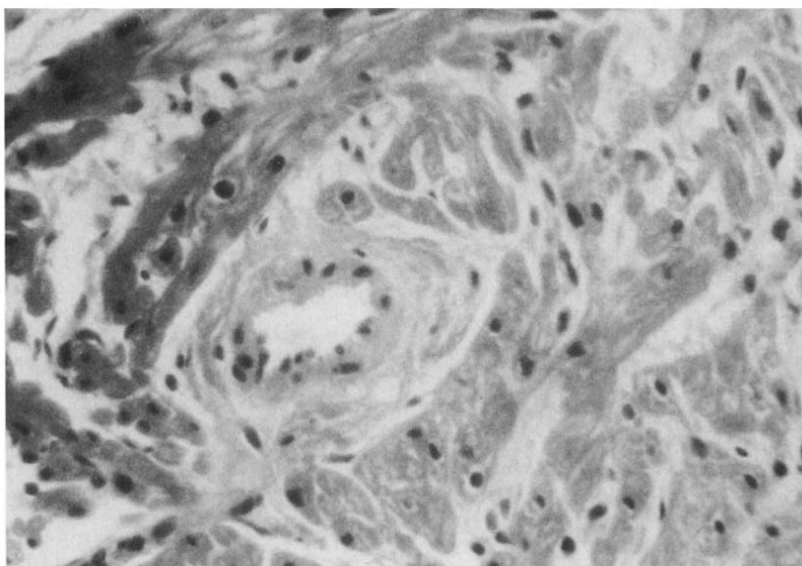
Thirty-seven biopsies were performed beginning 6 months after transplantation because of clinical suspicion of rejection or as a scheduled quarterly procedure. Adequate biopsy tissue for histologic analysis was obtained in all

cases. Biopsy was performed because of clinically suspected rejection in eight patients. In five episodes of suspected rejection manifested by tachycardia and irritability, the biopsy sample was free of cellular infiltrate. In three patients, rejection was suspected because of an increase in left ventricular posterior wall and septal thickness. In these three cases, the biopsy sample demonstrated focal perivascular myocyte damage, interstitial edema and minimal cellular infiltrate (Fig. 2), consistent with vascular/humoral rejection. In 29 biopsies performed for routine scheduled quarterly follow-up in asymptomatic patients, the biopsies were free of evidence of rejection.

Group II. Thirty episodes of rejection were demonstrated in 291 biopsies (10%) performed in 18 patients. Rejection had been clinically suspected in only 7 (23%) of the 30 biopsies demonstrating cellular infiltrate (Fig. 3). Rejection was clinically suspected on 17 occasions, but only in 7 (41%) of these 17 episodes did the biopsy sample demonstrate an acute cellular infiltrate. The results of the statistical analysis of rejection surveillance through clinical and biopsy methods for Groups I and II are presented in Table 1.

Safety evaluation. In Group I, the procedures were complicated in 1 (3%) of 37 biopsies, and the patients were

Figure 2. Endomyocardial biopsy tissue from a neonate, demonstrating endothelial cell swelling, interstitial myocardial edema and minimal lymphocyte infiltrate.



discharged 6 to 8 h after cardiac catheterization and biopsy. One patient developed a pneumothorax after attempted intravenous access by the internal jugular approach and required tube thoracostomy and overnight monitoring. There were no episodes of new tricuspid regurgitation, ventricular perforation or hemopericardium detected by echocardiography. In Group II patients, there were no complications. Two patients who underwent heart transplantation at age 1 year are now 3 years posttransplantation and have each had >20 biopsies without loss of venous access from the right femoral vein.

Discussion

Utility of biopsy. This study demonstrates the importance of endomyocardial biopsy for evaluation of clinically suspected rejection episodes in neonates and for routine rejection

surveillance in children after heart transplantation. In neonates (Group I) with clinically suspected rejection, endomyocardial biopsy identified which patients did not require rejection therapy. In those neonates without clinical signs of rejection, endomyocardial biopsy did not identify any rejection episodes, thus demonstrating that noninvasive measures alone appear to be sufficient to exclude rejection. Although noninvasive rejection surveillance assessing heart rate and echocardiographic left ventricular dimensions and function was valuable in the initial stages of patient evaluation, these variables could not be relied on for exact diagnosis of rejection and institution of therapy. Similar findings were described by Canter et al. (18), who found that in 10 episodes of clinically suspected rejection, results of all 10 biopsies were negative. However, the number of patients in both their series and our series was small.

In children (Group II), noninvasive rejection surveillance

Figure 3. Endomyocardial biopsy tissue from an asymptomatic child with no clinical or echocardiographic signs suggesting rejection, demonstrating grade 3 rejection characterized by dense lymphocytic infiltrate with evidence of myocyte damage.

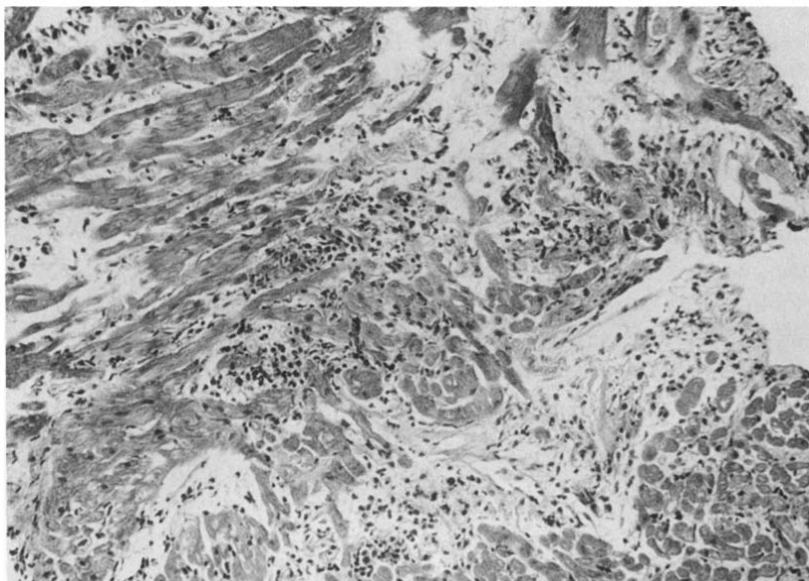


Table 1. Sensitivity of Clinical Suspicion of Rejection With Respect to Biopsy Findings

	Positive Biopsy Result	Negative Biopsy Result (95% CI)
Group I (neonates)		
Clinically suspected (no.)	3	5
Periodic surveillance (no.)	0	29
Proportion of rejection episodes detected by periodic surveillance	0%	0%-63%
Proportion of periodic surveillance "identifying" rejection	0%	0%-12%
Proportion of clinically suspected rejection confirmed by biopsy	38%	16%-75%
Proportion of procedures with complication	3%	0%-12%
Group II (children)		
Clinically suspected (no.)	7	10
Periodic surveillance (no.)	23	251
Proportion of rejection episodes detected by periodic surveillance	77%	61%-91%
Proportion of periodic surveillance "identifying" rejection	8%	5%-12%
Proportion of clinically suspected rejection confirmed by biopsy	41%	23%-67%
Proportion of procedures with complication	0%	< 1.8%

was less reliable than in neonates. In asymptomatic children, routine surveillance biopsy detected acute rejection in 8.4% of cases. Biopsy confirmed rejection in <50% of cases of clinically suspected rejection.

We demonstrated that repeated endomyocardial biopsy is a safe procedure (with only one complication in >330 procedures) that provides adequate tissue yield for accurate assessment of cellular rejection and myocyte damage. Biopsy therefore permits the precise documentation of the presence or absence of rejection, and consequently allows for titration of immunosuppressive agents to avoid their known side effects. Similarly, it avoids the risks of infection associated with excessive pulse steroid therapy if management is based on clinical signs alone.

Endomyocardial biopsy also provides tissue for analysis of humoral (vascular) rejection, as described by Hammond et al. (19). In 16 of 36 patients they found humoral rejection that was associated with significantly decreased survival. They concluded that immunofluorescence should be routinely performed on all biopsy specimens during the early months after transplantation. We found similar histologic and immunofluorescence findings in three neonatal transplant recipients. Prospective analysis of the relation of immunoglobulin and complement deposits in the coronary microvasculature may further define the relation of humoral rejection to accelerated graft coronary artery disease (20).

Echocardiographic surveillance. Various echocardiographic measures for detecting rejection have been studied in adult patients after heart transplantation. These indexes

are based on the premise that rejection results in abnormalities in diastolic function due to decreased left ventricular compliance from interstitial edema, cellular infiltrate and myocyte necrosis. Valantine et al. (21) utilized Doppler echocardiography to measure isovolumetric relaxation time and mitral valve pressure half-time after transplantation in 22 patients. They found a progressive decrease in isovolumetric relaxation time and a decrease in pressure half-time during biopsy-proved mild (cellular infiltrate) and moderate (myocyte necrosis) episodes of rejection. Desruennes et al. (22) found a similar decrease in isovolumetric relaxation time and pressure half-time correlated with rejection on endomyocardial biopsy. After treatment of the rejection episode with corticosteroids, the Doppler flow indexes returned to baseline values. However, in a subsequent study by Valantine et al. (23), recipient atrial contraction during systole was found to also shorten isovolumetric relaxation time and pressure half-time. This variation in timing of recipient atrial activity and the faster heart rates at rest in young children make these noninvasive Doppler studies less reliable and we (24) have not found them to be helpful.

Other surveillance methods. Cytoimmunologic monitoring of lymphocyte activation during cardiac allograft rejection has been reintroduced with the application of flow cytometry and monoclonal antibody markers. In previous studies by Fieguth et al. (25) in which lymphoblasts and prelymphoblasts were characterized, the overall sensitivity was 76% and specificity 79% compared with endomyocardial biopsy. This method became less useful as time progressed after transplantation. Analysis of these techniques during infectious episodes revealed activation of lymphocytes no different from that seen in rejection episodes. They concluded that cytoimmunologic monitoring is a useful adjunct to the biopsy procedure but that it could not replace routine surveillance biopsies.

Newer noninvasive surveillance modalities include tissue characterization of the myocardium by echocardiography and radionuclide indium-111 antimyosin imaging. Preliminary animal and human studies suggest that these new techniques (26-28) provide identification of severe rejection episodes associated with myocyte damage but are less reliable in mild or moderate episodes.

Conclusions. On the basis of our experience in neonatal transplant recipients with clinically suspected rejection, endomyocardial biopsy identified which patients did not require rejection therapy. Noninvasive measures alone appear to provide sufficient rejection surveillance in neonates without clinical signs of rejection. In children, noninvasive rejection surveillance was less reliable even in asymptomatic patients, suggesting that endomyocardial biopsy should be utilized. Endomyocardial biopsy is a low risk procedure that provides an accurate determination of rejection, and therefore appropriately guides immunosuppressive therapy after heart transplantation.

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